that both Pcs1/Mde4's clamping activity and its role as a condensin recruiter are required to efficiently prevent merotelic kinetochore attachments. Further studies are needed to unveil molecular details of how kinetochore pools of Pcs1/Mde4 and condensin complexes prevent merotely. Given the importance of this process for our understanding of how cells ensure faithful segregation of chromosomes, it is likely that this will continue to be an area of intense research in the future.

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DOI: 10.1016/j.cub.2011.06.059

Circadian Rhythms: *FLOWERING LOCUS T* Extends Opening Hours

Plants are more sensitive to light in the day than at night due to the circadian clock. The protein that acts downstream from the clock to modulate blue light signalling in stomata comes as a surprise; it is FT, which is thought to be the long-distance regulator of flowering.

Katharine E. Hubbard and Alex A.R. Webb

In Cambridge University, where we write, Francis Darwin, son to a famous father, studied the daily rhythms of stomatal movements and found stomata opened greater in response to light in the day than at night [1]. We now know that this is an example of rhythmic sensitivity to light due to modulation by the oscillator of the circadian clock, a process known as 'circadian gating' [2]. In a recent issue of *Current Biology*, Kinoshita *et al.* [3] report mechanisms involving *FLOWERING LOCUS T* (*FT*) that might permit circadian and photoperiodic regulation of stomatal sensitivity to blue light.

The stomatal pore provides an interface between the plant and the atmosphere through which CO_2 can enter the leaf to act as a substrate in photosynthesis, while at the same time H_2O is lost by evapotranspiration. Regulation of guard cell movements by the circadian oscillator conserves water by favouring stomatal opening in the morning, when ambient temperatures are low and promoting stomatal closure long before dusk to prevent water loss in the heat of the afternoon [4,5]. Mutant *Arabidopsis* lines with a compromised circadian oscillator have increased water loss during the day when compared to wild-type plants [5,6].

Stomatal aperture is ultimately regulated by the guard cell plasma membrane potential. In the morning, blue light and the circadian clock activate an electrogenic proton-pumping ATPase through a 14-3-3 protein-dependent pathway. The resulting H^+ efflux hyperpolarises the plasma membrane up to –250 mV, creating a driving force for the influx of K^+ through the KAT1 channel. K^+, along with Cl^- and malate, accumulate in the vacuole, resulting in water influx and an increase in guard cell turgor that opens the stomatal pore. Stomatal closure is brought about by inhibiting the H^+-ATPase and by Ca^{2+}- and OPEN STOMATA 1 kinase-dependent activation of SLOW ANION CHANNEL1 (SLAC1) to promote prolonged Cl^- efflux, which depolarises the plasma membrane. At plasma membrane potentials positive of –120 mV, the GUARD CELL OUTWARD RECTIFIER K CHANNEL opens, permitting K^+ efflux [7]. Loss of K^+, Cl^- and malate results in water efflux from the guard cell and stomatal closure.

Kinoshita *et al.* [3] provide evidence for a role in the regulation of stomatal aperture for *EARLY FLOWERING 3* (*ELF3*) and *FT*, genes that are more

normally associated with the photoperiodic control of flowering. Through screening for a suppressor of a closed-stomata phenotype in *phot1 phot2* double phototropin blue light receptor mutants that inhibit H^+-ATPase activity and stomatal opening, Kinoshita *et al.* [3] discovered a new allele of the circadian-associated gene *ELF3.* The allele, *elf3*-201, caused increased stomatal aperture, increased FT expression in guard cells and early flowering as would be expected from other *elf3* alleles [8]. To explore the significance of changes in *FT* expression in guard cells, Kinoshita *et al.* [3] investigated the effect of *ft* mutations on stomatal aperture, finding that *FT* over-expressors had open stomata, while *ft*-1 loss of function resulted in reduced aperture and no blue light activation of the H^+-ATPase. Similarly, *elf3*-201 had high ATPase activity, independent of light quality, and elevated 14-3-3 binding to the H^+-ATPase. The total amount of H^+-ATPase protein remained constant between the different mutant lines and treatments, indicating a post-translational mechanism is responsible for the effects of FT and ELF3 on pump activity. Kinoshita *et al.* [3] conclude that FT is a positive regulator of stomatal opening that modulates the blue light signalling pathway responsible for regulation of the H+ ATPase rather than direct alteration of the pump activity itself. Possibly because FT is a transcription factor, it could be altering the expression level of components of the blue light signalling network.

ELF3 was one of the earliest discovered circadian-associated genes in *Arabidopsis* [9,10]. Originally thought to modulate light input into the central oscillator, ELF3 has been recently demonstrated to bind to the promoter of the circadian oscillator gene *PSEUDO RESPONSE REGULATOR 9* and ELF3 is now considered to act as a component of the circadian oscillator that is required for the day-to-night transition of the circadian clock [11,12]. The circadian regulation of *ELF3* and expression in the guard cell make it a plausible candidate for a component of the network regulating stomatal behaviour.

Kinoshita *et al.* [3] propose that FT acts downstream of ELF3 to modulate blue light signalling (Figure 1). FT has

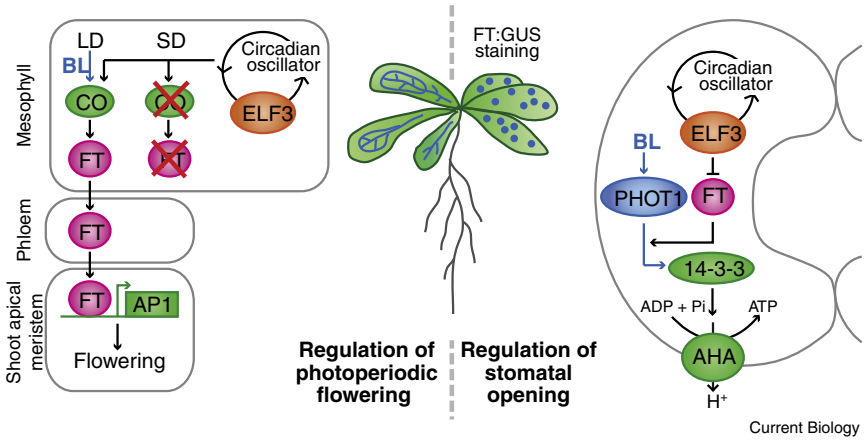

Figure 1. A new function for FT in stomata.

In addition to regulating flowering time, Kinoshita *et al.* [3] propose a role for FT in regulating stomatal aperture in *Arabidopsis*. In the control of photoperiodic flowering time, the circadian clock, including ELF3, controls the expression of CO, which is stabilised in the light in long days and promotes FT expression. FT travels through the phloem to the shoot apical meristem where it induces expression of the meristem identity genes such as *AP1*. In the model of Kinoshita *et al.* [3], the circadian clock, including ELF3, represses FT, which modulates the blue light signalling pathway that activates the guard cell plasma-membrane H^+-ATPase in a 14-3-3 protein-dependent manner. H^+-ATPase activity hyperpolarises the plasma membrane to provide a driving force for K^+ influx and stomatal opening. LD, long day; SD, short day; BL, blue light.

previously been considered to be primarily associated with the regulation of photoperiod-dependent flowering [13]. The expression of FT in *Arabidopsis* is induced by CONSTANS (CO) through coincidence between the circadian clock and the external photoperiodic cycle. *CO* mRNA oscillates with a circadian period, peaking 12–16 hours after dawn. In short days, the peak of *CO* abundance coincides with the dark and the CO protein is rapidly targeted for degradation, but in long days the peak of *CO* coincides with the light in which CO protein is stabilised and the levels of CO reach sufficient levels to promote *FT* expression. FT moves through the vasculature to the meristem to induce flowering [13]. Kinoshita *et al.* [3] determined that stomatal aperture was independent of whether a plant had flowered; therefore, it seems that FT is able to regulate two completely distinct physiological responses.

The functional significance of the findings of Kinoshita *et al.* [3] are not yet clear. Analysis of the temporal expression patterns of *ELF3* and *FT* support their coordinated activity, with both being maximally expressed towards the end of the day [14], when stomata are closing. The coordinated expression of both ELF3 and FT is difficult to reconcile with their opposing effects on blue light regulation of the guard cell H^+-ATPase. However, the above temporal dynamics are assumed on the basis of whole plant studies and Kinoshita *et al.* [3] present data showing *FT* expression is restricted to the vasculature and guard cells. Possibly the temporal dynamics of *ELF3* and *FT* expression is different in the guard cell compared to the vasculature, since cell-specific oscillators are present in plants and the vasculature has a specialised circadian clock [15,16].

The results of Kinoshita *et al.* [3] also identify a potential mechanism by which the circadian clock could modulate signalling pathways other than those directly regulated by light in the guard cell. The sensitivity of stomata to extracellular signals such as cold [17] and the drought-related hormone abscisic acid (ABA) [18] also depends on the time of day. It has been proposed that circadian regulation of stomatal sensitivity to ABA can be affected by the circadian oscillator gene *TIMING OF CAB1*, which reduces expression of the putative ABA receptor ABAR [19]. The findings of Kinoshita *et al.* [3] suggest an alternative mechanism by which the circadian clock could modulate stomatal responses to extracellular signals by providing a route through which the circadian clock component ELF3 modulates membrane potential

and therefore stomatal behaviour. A detailed study of the effect of *elf3* mutations and *FT* misexpression on ABA sensitivity might be warranted. However, regulation of H^+-ATPase activity is likely to be most important in the control of stomatal opening rather than closure, because during stomatal closure the activity of SLAC1, rather than the H^+-ATPase, will dominate the membrane potential. This might explain why *elf3*-201 mutants closed in response to high ABA [3]. The involvement of FT in blue light regulation of the H^+-ATPase could also suggest a photoperiodic-sensitive seasonal regulation of the H^+-ATPase, consistent with reports that there is a reduction in guard cell H^+ pumping activity in winter [20]. If circadian modulation of blue light regulation of H^+-ATPase activity, and hence membrane potential, occurs also in other cells, it could explain why the magnitude of cold-induced increases in $[Ca^{2+}]_{cyt}$ are gated by the circadian oscillator, because much of the cold-induced increase in $[Ca^{2+}]_{cyt}$ is thought to be due to influx across the plasma membrane and thereby sensitive to the plasma membrane potential [17].

The study of Kinoshita *et al.* [3] provides a testable hypothesis for the circadian gating of light signalling. Understanding the potentially antagonistic effects of the CO-regulated ELF3 and FT on H^+-ATPase activity will require identification of the downstream targets that interact physically with the H^+-ATPase. The cell-autonomous behaviour of symplastically isolated guard cells also point to the need for understanding circadian behaviour in single cell types to fully appreciate the impact intracellular circadian clocks have on the daily lives of plants.

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DOI: 10.1016/j.cub.2011.06.058

DNA Replication: Mammalian Treslin–TopBP1 Interaction Mirrors Yeast Sld3–Dpb11

There are many parallels between DNA replication in yeast and humans. Now, two recent studies extend this relationship by dissecting key conserved interactions necessary for initiation of the replisome.

Adam C. Mueller*, Mignon A. Keaton, and Anindya Dutta

Initiation of DNA replication involves an intricate cascade of cell cycle dependent steps of protein recruitment and activation. The process begins in early G1 phase when the ORC proteins localize to potential origins of replication. ORC then recruits Cdc6 and Cdt1, which facilitate the loading of the MCM2-7 replicative helicase complex to form a pre-replicative complex at a licensed origin. In order for replication to occur only once, loading of these factors onto chromatin must be temporally separate: ORC, Cdc6, Cdt1 and the MCM complex can only be loaded onto DNA in the absence of Cyclin dependent kinase (CDK) activity during G1 [1]. Activation of the MCM complex and formation of the active helicase requires the recruitment of the replication proteins Cdc45 and GINS complex to origins, which occurs when CDK activity rises to high levels at the transition to S phase [2]. While much is known about how the elevated CDK activity deters the licensing of origins that have already fired to prevent re-replication, until recently the mechanism by